The Investigation of the Effects of Low Light Laser Therapy on Insulin Secretion in Porcine Islets, a Pilot Study

Darren Raptis, SPT Angelo State University, San Angelo TX

Abstract

LED light technology is used in this pilot study to demonstrate the ability to create a cost effective light apparatus using 1-watt LEDs with wavelengths of 740nm,850nm, and 940nm to test for an increased insulin response using porcine islet of Langerhans cells. This is building on recent research showing increased insulin response in rat islet cells when exposed to LED light. However, due to the high variance and low sample size, statistically significant changes were not measured when irradiating cells with 740nm, 850nm, and 940nm LED light using 7.9 J/cm^2 and 15.8 J/cm^2 dosages along with low and high glucose conditions. There are however possible trends of increased insulin secretion that may become significant with increased sample size. There is a need to repeat this study to definitively determine if increased insulin secretion does occur in porcine islet of Langerhans cells along with any potential negative effects and what the optimal wavelengths and dosages would be. Using the cost effective light apparatus, additional wavelengths can be experimented with to cover between 300nm to over 1600nms. The information garnered from this research has the potential to improve islet of Langerhans cell transplantation for type one diabetes (T1D) patients by decreasing the number of cells needed to be therapeutic and increasing insulin release.

Introduction

Many portions of the electromagnetic spectrum are harnessed by humankind for an extensive range of beneficial applications. Some of the most notable examples are cell phone signals, microwave ovens, light emitting diodes (LED), radiographs, diagnostic ultrasound machines, CT scanning machines, and MRI machines. LED light technology, in particular, is

currently in a pioneering stage with light output efficiency doubling every 18-24 months since 1968.[1] This has allowed economy of scale and reduced cost to reach scientists for experimentation.[2] Some current notable uses of LED lighting for medical treatment include using light for skin disorders,[3] seasonal depression,[4] circadian rhythm sleep disorders,[5] and neonatal jaundice.[6] Physical therapists have been using the technology to help with tissue healing,[7-12] pain management,[13-17] and treating joint disorders.[18-20]

There is considerable research interest in this field for the treatment of medical conditions either as an adjunct to medication or on its own. For example, light irradiation has been shown effective in stimulating protein release from secretory cells such as mast cells,[21-22] macrophages,[23] keratinocytes,[24] thymus cells,[25] smooth muscle cells, fibroblasts, and cardiomyocytes.[26] Islet of Langerhans are clusters of cells found in the pancreas. They contain beta cells which produce insulin to regulate blood glucose in the human body. Light irradiation has recently been shown to improve islet function in rats using red light (630nm) or infrared light (810nm) to significantly increase insulin secretion after glucose challenge tests.[27,28] This information suggests the possibility for light therapy to aide in type one diabetes (T1D) treatment.

According to the CDC's Diabetes Report Card and National Diabetes Statistics Report, nearly 208,000 people have T1D under the age of 20 as of 2014.[29] T1D results from the pt.'s body destroying its own insulin secreting cells contained in the islets of Langerhans. One promising experimental treatment under current investigation is islet transplantation. By receiving healthy insulin producing islet cells from a donor(s), T1D patients may no longer need insulin injections.[30-33] However, there are still significant challenges preventing this treatment method from changing over from an experimental procedure.[30,34-38] The most notable challenges

are the loss of healthy islets after transplantation caused by an autoimmune response from the recipient, the shortage of organ donors, and the need for two deceased human pancreases to obtain enough islets to achieve a normal glycemic level for one T1D patient.[36,39]

New LED technology could be used to benefit type one diabetes patients and this evolving new medical procedure. Low intensity infrared light modulation of biological processes of the human body is coined photobiomodulation. The wavelength of light used affects the depth of tissue penetration and ultimately the cellular effects.[40] Light with a longer wavelength penetrates deeper than light with a shorter wavelength.[41,42] Currently, there is relatively little research on photobiomodulation using infrared light to evoke an insulin response from islet of Langerhans cells.

The purpose of this research is to perform a pilot study to demonstrate how to cost effectively construct a 1-watt LED light apparatus and to determine if light at wavelength of 740nm, 840nm, and 940nm is able to evoke an insulin secretion response from isolated porcine islet of Langerhans cells.

Methodology

Research Design

The research design chosen for this project was a pilot study format. The reasons are to test for feasibility of designing an LED light apparatus along with modifying existing islet isolation and irradiation protocols for porcine islets. This pilot study should justify expanding research.

Materials

Tools: Hot glue gun, Soldering gun, Scissors, Wire striper and cutter,

Materials to construct LED light Base: Retort stand with ring clamp, 4mm thick opaque plastic 4” by 4” sheet

Materials to construct LED light: LED driver model Chihui CH031 with specifications (LED driver specifications: input AC 85V- 255V at 50/60 Hz, output: constant current 320mA DC 2.5-12V), 1 LED 940nm 1-watt infrared, 1 LED 850nm 1-watt infrared, 2 LED 760nm 1-watt red, 1 6ft IEC power cord, Velcro 6” by 4” cut to fit, 4 pairs of 22AWG Wire JST SM 2P Connectors Male and Female, and 6 feet of 24 AWG two conductor power wire.

Bonding materials needed: All purpose hot glue sticks, 6 inch zip ties, Solder spool, and Electrical tape.

Note: All LED parts were purchased from EBay. The remaining materials can be purchased online or from a big box home supply store.

Procedures

Constructing LED light apparatus

First, acquire all materials and tools needed to construct the LED light system and gather them to a suitable working site such as a table top. Begin by making the detachable experimental LEDs. First, Solder the red (+) and black (-) wires from the JST SM 2P female connector to the (+) and (-) labeled soldering points on the LED using the soldering gun. Next, wrap the soldered wires around to the bottom of the LED base and attach using hot glue. From there, cut out Velcro, hook surface side, into a donut shape and affix to the top of the LED surface leaving a hole just large enough for the LED to pass through. The Velcro comes with an adhesive backing that can be further secured using some hot glue. See Figure 1-2 Repeat this procedure to complete each wavelength LED. There should be a 740nm, 850nm, and 940nm LED when complete. Set these aside to begin working on the LED Light base.

Figure 1. Front LED exchange bulb light

Figure 2. Back LED exchange bulb light

To begin constructing the light base retrieve the retort stand and base and secure the LED driver to the stand using zip ties. Next, take the opaque plastic sheet and use the scissors to modify the sheet to the size of the ring portion of the stand. Attach the plastic sheet using hot glue. Finish by cutting a 1cm diameter hole in the middle of the plastic sheet. Next cut out loop side Velcro and attach it to the bottom surface of the plastic sheet around the hole without covering it. This will be where the exchangeable LEDs will be attached and removed. The petri dishes sit on top of the opaque plastic sheet centered to the LED to irradiate the cells.

The last portion of the build is to wire the LED light apparatus. First, take the 6 ft. IEC power cord and cut the female plug off. There should be 3 wires exposed (black, red, green). Next, cut away the green wire and then strip back ½" of the copper wire sheathing from the red and black wire. Twist the red exposed wire from the 6 ft. power cable to one of the two power input wires from the LED driver. It does not matter which wire. Solder the wire to secure in place. Now, wrap newly soldered wire with electrical tape. Next, twist the black exposed wire from the 6 ft. power cable to the second of two power input wires from the LED driver. Solder the twisted black and power input LED driver wires together. Wrap the newly soldered wire with electrical tape. The LED driver should now have a power cord attached to it. See figure 3. The

Figure 3. Finished attaching power cord to LED driver

last portion of the wiring completes the quick connector for changing the LEDs and an indicator LED to inform the experimenter that the apparatus is on or off. First, wire a male SM 2P quick connector and 760nm red LED in series with enough wire to place the red LED at the bottom of the light apparatus along with enough wire to attach and remove different wavelengths of experimental LEDs to the light Velcro base constructed through a JST SM 2P male connector. The wire should begin with the positive LED driver output wire attached to additional wire for length. Following the wire should connect to the positive end of the JST SM 2P male connector. The wire loop continues down the black (-) wire of the JST SM 2P male connector to additional wire for length. From there, the wire should continue to the positive terminal of the red 740nm LED. A second wire should then connect to the negative terminal of the same 740nm red LED. This wire should end by attaching to the negative output wire of the LED driver completing the loop. Each connection of wire should be connected by stripping the insulation ¼" back and soldering the connections. End with insulating each connection by wrapping with electrical tape. See completed apparatus in figure 4.

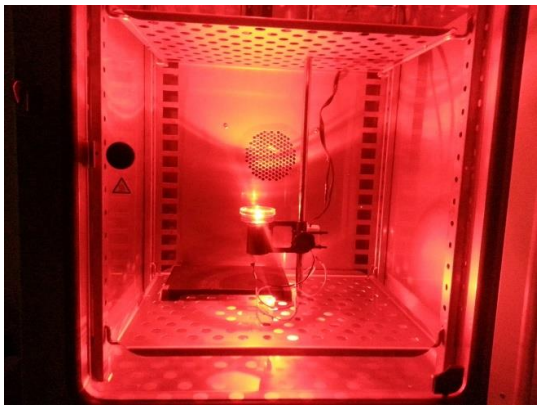


Figure 4. Showing the completed LED apparatus within an incubation chamber

Harvesting and isolating Porcine pancreas islet of Langerhans cells

Porcine pancreas tissue was isolated from animals harvested for food and fiber education research. For this pilot study, five pig donors were harvested humanely according to the United States Department of Agriculture (USDA) guidelines at ASU's Food Safety and Product Development Laboratory. The tissue samples were collected individually with the warm ischemia time, the time between the cessation of the heart beating and the placement of the pancreas tissue into the preservation solution, was within 30 minutes. The pancreas tissue was stored in 4 ^{0}C Belzer UW Cold storage solution and transported on ice to ASU's Diabetes Research Lab for islet isolation. The cold ischemia time, the time between the placement of the pancreas into the Belzer UW Cold Storage solution and the start of islet isolation, was within 2 hours. Islet isolation was conducted using a modified Ricordi method.[43] The main components of the isolation were soaking the pancreas tissue in a solution of Liberase DL (1.0 mg/mL; Sigma-Aldrich) and letting it digest in a sterilized 37 ^{0}C incubation chamber for 20-30 minutes with occasional gentle shaking. The digested tissue was then collected into RPMI-1640 medium with 10% fetal bovine serum (FBS) and washed twice to remove the digestive enzyme. The islets were then purified by using a continuous density gradient based on a modified protocol.[44] The islets were then extracted and placed into RPMI-1640 with 10% porcine serum and 1% antibiotic/antimycotic and put into a 37 ^{0}C culture chamber containing 5% CO2. The now isolated islet of Langerhans cells were then left to rest for 24 hours before being submitted to the light and glucose trials.

LED light irradiation on islets

The isles were transferred into a 24-well plate with low (3mM) or high (16.7 mM) glucose and subjected to a 1-watt LED light with a wavelength of either 740nm, 850nm, or 940nm for 15

seconds (7.9 J/cm^2) or 30 seconds (15.8 J/cm^2) in a 37 ^{0}C incubator. After a 60-minute incubation period, the supernatant was collected and frozen at -80 ^{0}C. The samples were then analyzed by an insulin enzyme-linked immunosorbent assay (ELISA) test kit to measure insulin secretion.

Statistical Analysis

The samples were compared to the control using one-way ANOVA statistical analysis

Results

The raw data was compiled and organized into tables 1-3 The data for Tables 2 and 3 were normalized by comparing insulin secretion to total protein content by a relative fold change using the control sample. Each sample contained a minimum of 100 islets. Table 1 shows the change in insulin secretion in response to no LED irradiation and LED irradiation for one porcine subject. The data shows that low dose light (7.9 J/cm^2) increased insulin production under high glucose while the high dose light (15.8 J/cm^2) increased insulin production under the low glucose level.

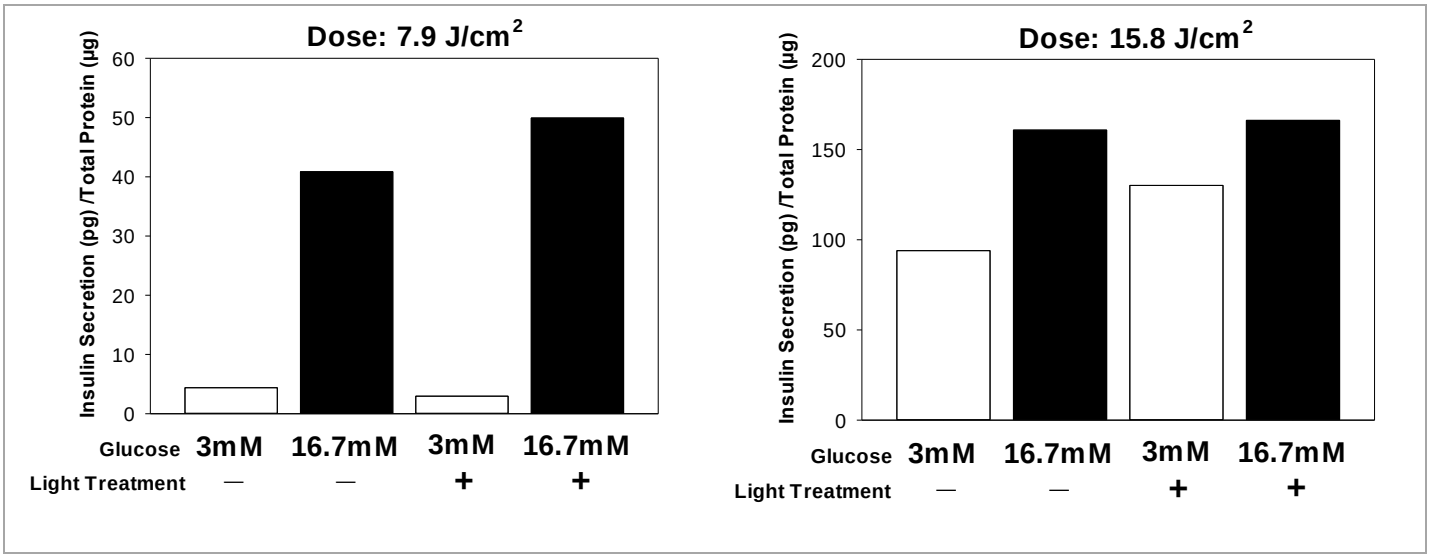
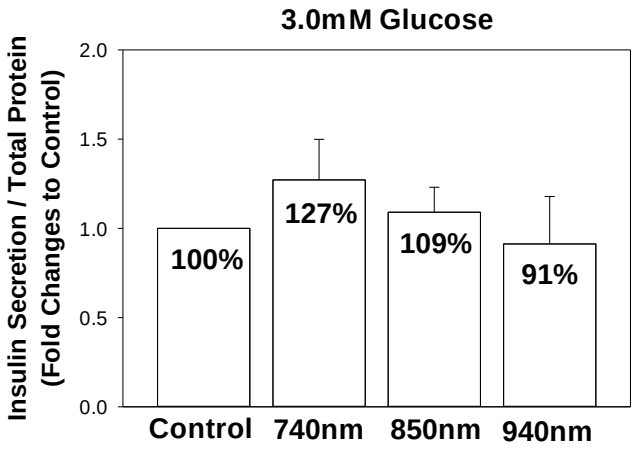


Table 1. Insulin secretion after light irradiation (1-Watt 740nm LED). Two different doses were applied for the two trials respectively. Islets were isolated from one pig in each trial with at least 100 islets in each

For the next portion of the study, islet from five pigs were treated by three different wavelength 1-watt LEDs (740nm, 850nm, and 940nm) under 3.0mM and 16.7mM glucose seen in Table 2. When compared to the controls that received no light irradiation insulin secretion under low glucose light irradiation, Table 2a, increased 27% using 740nm and 9% using 850nm. Using 940nm light irradiation insulin secretion decreased 9%. In Table 2b high glucose, light irradiation using 740nm increased insulin secretion 36%, but decreased 38% under 850nm and 30% under 840nm. Overall, the differences were not statistically significant due to small sample size and high variation between trials.



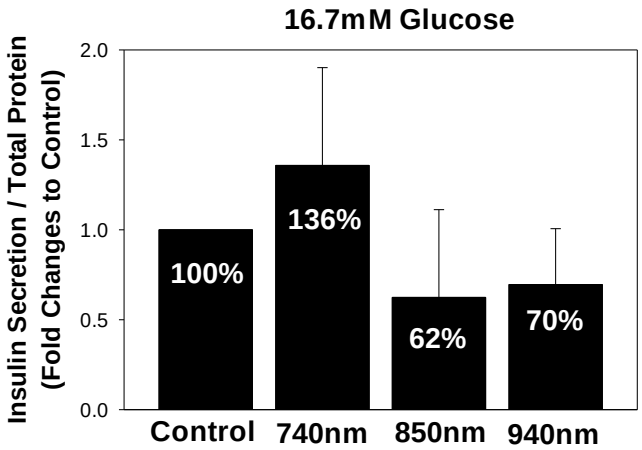


Table 2. Insulin secretion after light irradiation with a dose of 15.8 J/cm^2 under low and high glucose. N = 5 trials in each group. Each trial contained at least 100 islets from one pig. The expressions in each group are presented as relative fold difference to the control group. The left portion of the table is 2a and the table to the right is 2b.

In Table 3, at 740nm light irradiation under low glucose (3.0mM) there is a possible upward trend in insulin secretion relative to the control. With a 7.9 J/cm^2 dose there is a 19%

increase in insulin secretion relative to the control and a 27% increase at the 15.8 J/cm^2 dosage level. When the islets were bathed in high glucose (16.7mM), the 7.9 J/cm^2 dose showed a 2% increase in insulin secretion while the 15.8 J/cm^2 dose showed a 36% increase in insulin secretion relative to the control. At 850nm light irradiation under low glucose (3.0mM) the 7.9 J/cm^2 dose shows a 10% increase in insulin secretion relative to the control and a 9% increase at the 15.8 J/cm^2 dosage level. When the islets were bathed in high glucose (16.7mM) the 7.9 J/cm^2 dose showed a 28% decrease in insulin secretion while the 15.8 J/cm^2 dose showed a 38% decrease in insulin secretion relative to the control. At 940nm light irradiation under low glucose (3.0mM) the 7.9 J/cm^2 dose shows a 2% decrease in insulin secretion relative to the control and a 9% decrease at the 15.8 J/cm^2 dosage level. The standard error is 43%. When the islets were bathed in high glucose (16.7mM) the 7.9 J/cm^2 dose showed a 58% decrease in insulin secretion while the 15.8 J/cm^2 dose showed a 30% decrease in insulin secretion relative to the control.

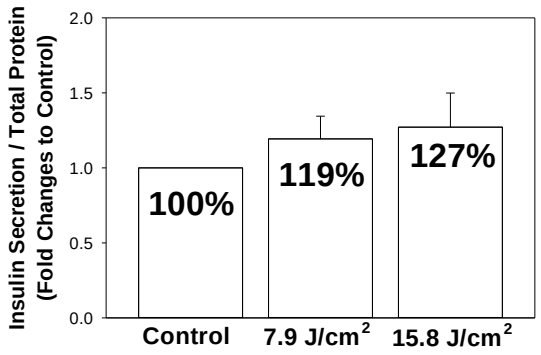
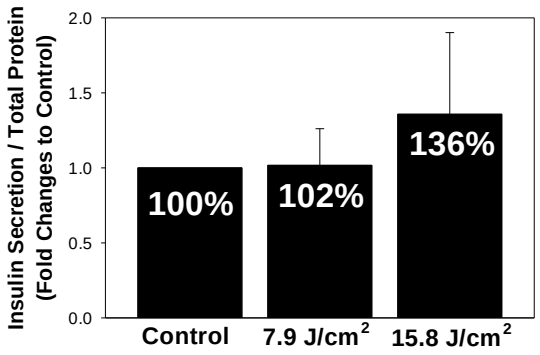
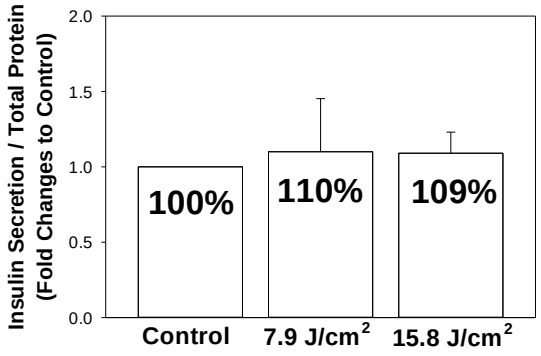
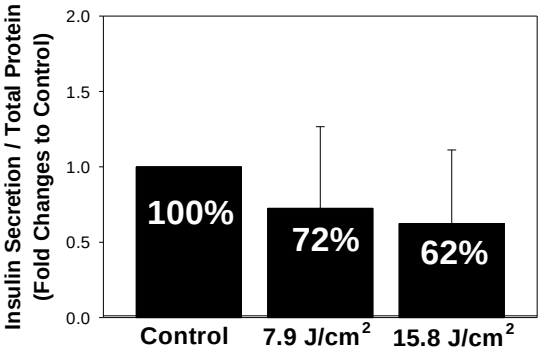
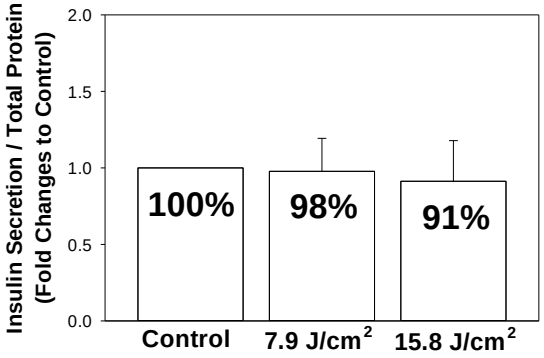
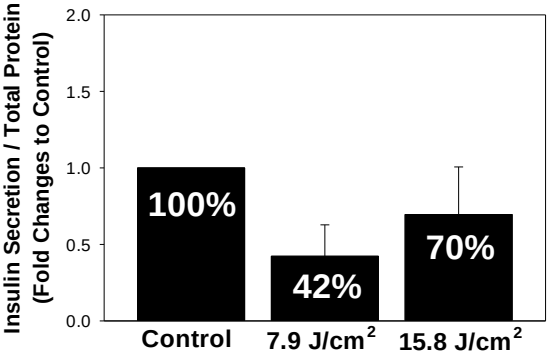
	3.0mM Glucose	16.7mM Glucose
740nm		
850nm		
940nm		

Table 3. Insulin secretion response using three different wavelengths and two different doses of LED irradiation in addition to low or high glucose conditions. N = 5 trials in each group. Each trial contained at least 100 islets from one pig. The expressions in each group are presented as relative fold difference compared to the control group.

Discussion

The purpose of this pilot study is to demonstrate the ability to construct a cost effective LED light apparatus and to create a modified porcine islet of Langerhans protocol to measure insulin secretion while modify light wavelength, light dosage, and glucose environment. The results of the pilot study do show a possible insulin secretion change that appears dependent on the light dose, and light wavelength. At 740nm of light, increasing light dosage shows a possible upward trend in insulin secretion under the low and high glucose environment. Also, when the wavelength of light changed to 850nm there is a possible upward insulin secretion trend with increasing light dosage in the low glucose environment. However, in the high glucose environment there is a possible downward trend of insulin secretion. Finally, the 940nm light shows a possible downward trend under low and high glucose environments. Overall, the insulin trends for all the results were not statistically significant due to high variation and small sample size. The pilot study does prove, however, that a modified islet isolation technique created for porcine tissue along with creating an LED light to test wavelengths and dosage does produce viable results to continue further research. By expanding the light wavelengths and dosages along with increasing sample size, the optimal wavelength and dosage can be discovered. Also the pilot study suggests there is the possibility of light irradiation creating an adverse effect in decreasing insulin secretion or causing cell death. Further research can discover potential wavelength and dosages to be avoided in the future using the experimentation methods created in this pilot study.

LED light technology is currently being used by physical therapists in the clinic for tissue healing [7-12] and pain management.[13-17] The equipment in the clinic most commonly uses light with wavelengths between 600 nm and 1300nm due to its optimal depth of penetration in human

tissue.[45,46] The equipment however is not specific enough for this experimentation because the wavelengths emitted are designed to deliver a broad wavelength range by using a mix of different LEDs.[47] LED equipment irradiating narrow bandwidths that could be used in experimentation to determine the most effective bandwidth and dosage for islet insulin production does not cheaply exist. The LED apparatus designed and created for this experiment costs under 40 dollars in parts and can be assembled in under 3 hours. In addition, the light apparatus is designed to quickly switch LEDs to experiment with narrow bandwidths from 300nm to above 1600nm. In addition, there is a second LED indicator built into the apparatus to provide visual real-time feedback to the experimenter when the light is on and that the LED used for the experiment is indeed functional. This is a cost effective indicator that serves as a check system to maintain high confidence that the apparatus is functional. If the LED being used for the experiment was non-functional then it would be indicated by the second visual check LED remaining unlit. For this experiment 740nm, 850nm, and 940nm 1-watt LEDs were used but could be expanded to cover 300nm to beyond 1600nm to determine the optimal dosage and wavelengths for insulin secretion.

Overall, the information discovered by expanding this research can be applied to the medical field through T1D islet transplantation research. Currently T1D patients receive insulin injections or an insulin pump to control their condition.[48] In extreme cases a T1D patient may receive a pancreas transplant if they are unable to control their glucose levels.[49] The problems with traditional transplantation are the complications and risks associated with major surgery. A viable minimally invasive procedure would lower the risk and create a more widely available choice for T1D patients. The most notable challenges for islet transplantation treatment at this time are the loss of healthy islets after transplantation caused by an autoimmune response, the

shortage of organ donors, inadequacy of vascularization or low oxygen tension in the liver, and the need for two deceased human pancreases to obtain enough islets to achieve a normal glycemic level 23.[36,39] Given the limits at this time, there are several original approaches to overcome these challenges. To address the autoimmune response, researchers are attempting to encapsulate islets through extravascular macrocapsulation and microencapsulation to prevent immune destruction after transplantation.[32,33] To address low vascularization/oxygenation, researchers are exploring transplanting islets to alternative sites such as the greater omentum, or intramuscular and subcutaneous spaces.[34,36] Finally, to minimize the need for additional islet tissue, LED irradiation treatment using optimal dosage and wavelength research can be used to increase insulin production of islets to reduce the number of islets needed for a successful transplantation. Light therapy has been demonstrated to penetrate tissue to a depth between a few millimeters to 50 millimeters, which is dependent on the wavelength applied.[41,42] Light protocols could be developed to irradiate islets located in the greater omentum or subcutaneous tissue to stimulate an increased insulin response. The outcomes of this research as an adjunct to the overall transplantation procedure could help transition it from experimental to a minimally invasive paid treatment in the near future.

Some potential areas of improvement on this pilot study design would be better control of the variation on tissue harvesting; specifically, the animal's gender may be a contributing variable to the variation seen between subjects. Also, increasing the sample size with additional funding could have produced a statistical change in insulin response and increased the validity of the pilot study beyond a proof of concept.

Conclusion

LED light technology has been shown to increase islet insulin secretion in rats. This pilot study demonstrates the ability to create a cost effective experimental light apparatus to test dosage and wavelength of LED light from 300nm to 1600nm along with a modified islet isolation protocol for porcine pancreatic tissue. The pilot study was able to demonstrate the feasibility of the research protocol and show a potential trend in stimulating an increased insulin response using porcine islets. However, due to the high variance and low sample size, statistically significant changes were not measured when irradiating cells with 740nm, 850nm, and 940nm LED light using 7.9 J/cm2 and 15.8 J/cm2 dosages and low and high glucose conditions. There is a need to repeat this study to definitely determine if increased insulin secretion does occur in porcine islet of Langerhans cells and what the optimal wavelengths and dosages would be. This information has the potential to improve islet of Langerhans cell transplantation for T1D patients by decreasing the number of cells needed to have a successful transplantation.

References

1. Steigerwald D, Bhat J, Collins D et al. Illumination with solid state lighting technology. *IEEE J Select Topics Quantum Electron*. 2002;8(2):310-320. doi:10.1109/2944.999186.
2. Morrow R. LED lighting in Horticulture. *HortScience*. 2008;47(7). Available at: http://hortsci.ashspublications.org/content/43/7/1947.full#cited-by. Accessed May 9, 2016.
3. Elman M, Slatkine M, Harth Y. The effective treatment of acne vulgaris by a high-intensity, narrow band 405-420 nm light source. J Cosmet Laser Ther. 2003;5(2):111-7.
4. Golden RN, Gaynes BN, Ekstrom RD, et al. The efficacy of light therapy in the treatment of mood disorders: a review and meta-analysis of the evidence. Am J Psychiatry. 2005;162(4):656-62.
5. Ancoli-israel S, Martin JL, Kripke DF, Marler M, Klauber MR. Effect of light treatment on sleep and circadian rhythms in demented nursing home patients. J Am Geriatr Soc. 2002;50(2):282-9.
6. Maisels MJ, Mcdonagh AF. Phototherapy for neonatal jaundice. N Engl J Med. 2008;358(9):920-8.
7. Kajagar BM, Godhi AS, Pandit A, Khatri S. Efficacy of Low Level Laser Therapy on Wound Healing in Patients with Chronic Diabetic Foot Ulcers-A Randomised Control Trial. The Indian journal of surgery. 2012 Oct;74(5):359-63.
8. Neiburger EJ. Rapid healing of gingival incisions by the helium-neon diode laser. Journal of the Massachusetts Dental Society. 1999 Spring;48(1):8-13, 40.
9. Tumilty S, McDonough S, Hurley DA, Baxter GD. Clinical effectiveness of low-level laser therapy as an adjunct to eccentric exercise for the treatment of Achilles'

tendinopathy: a randomized controlled trial. Archives of physical medicine and rehabilitation. 2012 May;93(5):733-9.

10. Tumilty S, Munn J, McDonough S, Hurley DA, Basford JR, Baxter GD. Low level laser treatment of tendinopathy: a systematic review with meta-analysis. Photomedicine and laser surgery. 2010 Feb;28(1):3-16.
11. Chang WD, Wu JH, Jiang JA, Yeh CY, Tsai CT. Carpal tunnel syndrome treated with a diode laser: a controlled treatment of the transverse carpal ligament. Photomedicine and laser surgery. 2008 Dec;26(6):551-7.
12. Iyomasa MM, Issa JP, de Queiroz Tavares ML, et al. Influence of low-level laser associated with osteogenic proteins recombinant human BMP-2 and Hevea brasiliensis on bone repair in Wistar rats. Microscopy research and technique. 2012 Feb;75(2):117-25.
13. Emanet SK, Altan LI, Yurtkuran M. Investigation of the effect of GaAs laser therapy on lateral epicondylitis. Photomedicine and laser surgery. 2010 Jun;28(3):397-403.
14. Carrasco TG, Guerisoli LD, Guerisoli DM, Mazzetto MO. Evaluation of low intensity laser therapy in myofascial pain syndrome. Cranio : the journal of craniomandibular practice. 2009 Oct;27(4):243-7.
15. Jovicic M, Konstantinovic L, Lazovic M, Jovicic V. Clinical and functional evaluation of patients with acute low back pain and radiculopathy treated with different energy doses of low level laser therapy. Vojnosanitetski pregled Military-medical and pharmaceutical review. 2012 Aug;69(8):656-62.
16. Rayegani S, Bahrami M, Samadi B, Sedighipour L, Mokhtarirad M, Eliaspoor D. Comparison of the effects of low energy laser and ultrasound in treatment of shoulder

myofascial pain syndrome: a randomized single-blinded clinical trial. European journal of physical and rehabilitation medicine. 2011 Sep;47(3):381-9.

17. Saayman L, Hay C, Abrahamse H. Chiropractic manipulative therapy and low-level laser therapy in the management of cervical facet dysfunction: a randomized controlled study. Journal of manipulative and physiological therapeutics. 2011 Mar-Apr;34(3):153-63.
18. Alfredo PP, Bjordal JM, Dreyer SH, et al. Efficacy of low level laser therapy associated with exercises in knee osteoarthritis: a randomized double-blind study. Clinical rehabilitation. 2012 Jun;26(6):523-33.
19. Beasley J. Osteoarthritis and rheumatoid arthritis: conservative therapeutic management. Journal of hand therapy : official journal of the American Society of Hand Therapists. 2012 Apr-Jun;25(2):163-71; quiz 72.
20. Meireles SM, Jones A, Natour J. Low-level laser therapy on hands of patients with rheumatoid arthritis. Clinical rheumatology. 2011 Jan;30(1):147-8.
21. Bayat M, Vasheghani MM, Razavie N, Jalili MR. Effects of low-level laser therapy on mast cell number and degranulation in third-degree burns of rats. Journal of rehabilitation research and development. 2008;45(6):931-8.
22. Silveira LB, Prates RA, Novelli MD, et al. Investigation of mast cells in human gingiva following low-intensity laser irradiation. Photomedicine and laser surgery. 2008 Aug;26(4):315-21.
23. Young S, Bolton P, Dyson M, Harvey W, Diamantopoulos C. Macrophage responsiveness to light therapy. Lasers in surgery and medicine. 1989;9(5):497-505.

24. Yu HS, Chang KL, Yu CL, Chen JW, Chen GS. Low-energy helium-neon laser irradiation stimulates interleukin-1 alpha and interleukin-8 release from cultured human keratinocytes. The Journal of investigative dermatology. 1996 Oct;107(4):593-6.
25. Novoselova EG, Glushkova OV, Cherenkov DA, Chudnovsky VM, Fesenko EE. Effects of low-power laser radiation on mice immunity. Photodermatology, photoimmunology & photomedicine. 2006 Feb;22(1):33-8.
26. Kipshidze N, Nikolaychik V, Keelan MH, et al. Low-power helium: neon laser irradiation enhances production of vascular endothelial growth factor and promotes growth of endothelial cells in vitro. Lasers in surgery and medicine. 2001;28(4):355-64.
27. Irani S, Mohseni Salehi Monfared SS, Akbari-Kamrani M, Ostad SN, Abdollahi M, Larijani B. Effect of low-level laser irradiation on in vitro function of pancreatic islets. Transplantation proceedings. 2009 Dec;41(10):4313-5.
28. De Meulenaer EC, Louchami K, Zhang Y, Bulur N, Sener A, Malaisse WJ. Effects of infrared light on insulin release, cationic fluxes and cellularity in insulin- producing cells. Met Func Res Diab 2009 2009/11/18;2:63-70.
29. National Diabetes Statistics Report, 2014. 1st ed. Atlanta: National Center for Chronic Disease Prevention and Health Promotion; 2014:1. Available at: http://webcache.googleusercontent.com/search?q=cache:iIuoBAZu8RUJ:www.cdc.gov/diabetes/pubs/statsreport14/national-diabetes-report-web.pdf+&cd=1&hl=en&ct=clnk&gl=us. Accessed May 9, 2016.
30. Hatziavramidis DT, Karatzas TM, Chrousos GP. Pancreatic islet cell transplantation: an update. Annals of biomedical engineering. 2013 Mar;41(3):469-76.

31. Barton FB, Rickels MR, Alejandro R, et al. Improvement in outcomes of clinical islet transplantation: 1999-2010. Diabetes care. 2012 Jul;35(7):1436-45.

32. Johnson PR, Jones KE. Pancreatic islet transplantation. Seminars in pediatric surgery. 2012 Aug;21(3):272-80.

33. Jamiolkowski RM, Guo LY, Li YR, Shaffer SM, Naji A. Islet transplantation in type I diabetes mellitus. The Yale journal of biology and medicine. 2012 Mar;**85**(1):37-43.

34. Khosravi-Maharlooei M, Hajizadeh-Saffar E, Tahamtani Y, et al. THERAPY OF ENDOCRINE DISEASE: Islet transplantation for type 1 diabetes: so close and yet so far away. European journal of endocrinology / European Federation of Endocrine Societies. 2015 Nov;**173**(5):R165-83.

35. Maffi P, Secchi A. Clinical results of islet transplantation. Pharmacological research. 2015 Aug;**98**:86-91.

36. Zinger A, Leibowitz G. Islet transplantation in type 1 diabetes: hype, hope and reality - a clinician's perspective. Diabetes/Metabolism Research and Reviews. 2013:n/a-n/a.

37. Danobeitia JS, Fernandez LA. Clinical islet transplantation: recent advances in the field. Clinical transplants. 2012:193-200.

38. McCall M, Shapiro AM. Update on islet transplantation. Cold Spring Harbor perspectives in medicine. 2012 Jul;**2**(7):a007823.

39. Vehik K, Dabelea D. The changing epidemiology of type 1 diabetes: why is it going through the roof? Diabetes Metab Res Rev. 2011 Jan;**27**(1):3-13.

40. Hawkins D, Houreld N, Abrahamse H. Low level laser therapy (LLLT) as an effective therapeutic modality for delayed wound healing. Annals of the New York Academy of Sciences. 2005 Nov;**1056**:486-93.

41. Esnouf A, Wright PA, Moore JC, Ahmed S. Depth of penetration of an 850nm wavelength low level laser in human skin. Acupuncture & electro-therapeutics research. 2007;**32**(1-2):81-6.

42. Hudson DE, Hudson DO, Wininger JM, Richardson BD. Penetration of laser light at 808 and 980 nm in bovine tissue samples. Photomedicine and laser surgery. 2013 Apr;**31**(4):163-8.

43. Matsumoto, S., et al., *Successful islet transplantation from nonheartbeating donor pancreata using modified Ricordi islet isolation method.* Transplantation, 2006. **82**(4): p. 460-5.

44. Van der Burg, M.P., I. Basir, and E. Bouwman, *No porcine islet loss during density gradient purification in a novel iodixanol in University of Wisconsin solution.* Transplant Proc, 1998. **30**(2): p. 362-3.

45. King PR. Low level laser therapy: a review. Low-Level-laser-Therapie: ein Uebersichtsbericht. 1990(3):141.

46. Kolari PJ. Penetration of unfocused laser light into the skin. Archives of dermatological research. 1985;**277**(4):342-4.

47. Cameron, M.H., *Physical agents in rehabilitation : from research to practice / [edited by] Michelle Cameron*. 2013: St. Louis, Mo. : Saunders/Elsevier, c2013. 4th ed.

48. Bergenstal RM, Tamborlane WV, Ahmann A, et al. Effectiveness of sensor-augmented insulin-pump therapy in type 1 diabetes. N Engl J Med. 2010;363(4):311-20.

49. Pancreas transplantation for patients with type 1 diabetes. Diabetes Care. 2000;23 Suppl 1:S85.